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Inhibition of ochratoxin A production and growth of *Aspergillus* species by phenolic antioxidant compounds

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Abstract The phenolic antioxidants, gallic acid, vanillic acid, protocatechuic acid, 4-hydroxybenzoic acid, catechin, caffeic acid, and chlorogenic acid were studied for their effects on ochratoxin A (OTA) production and fungal growth of ochratoxigenic Aspergilli. Of the 12 strains tested, which included *A. alliaceus, A. lanosus, A. ochraceus, A. albertensis, A. melleus, A. sulphureus, A. carbonarius, A. elegans*, and *A. sclerotiorum*, the greatest inhibition of OTA production was seen in *A. sulphureus, A. elegans,* and *A. lanosus*. Vanillic acid and 4-hydroxybenzoic acid were the most inhibitory to both OTA production and growth of most of the strains tested. However, *A. ochraceus* was not inhibited by either compound, and *A. carbonarius* was not inhibited by vanillic acid. The effect of each compound on OTA production and growth differed among strains and generally was variable, suggesting that species-specific OTA production and response to phenolic compounds may be influenced by different ecological and developmental factors. In addition, inhibition of OTA production by antioxidant compounds may be useful in determining biosynthetic and regulatory genes involved in both OTA production and stress response in ochratoxigenic Aspergilli.

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Introduction

Ochratoxin A (OTA) is a polyketide mycotoxin composed of a chlorinated dihydro-methyl-isocoumarin moiety linked via an amide bond to phenylalanine [1]. OTA is a secondary metabolite produced by several *Aspergillus* species, including but not limited to *A. ochraceus, A. alliaceus, A. auricomus, A. sulphureus, A. carbonarius, A. niger*, and *A. glaucus*, as well as *Penicillium verrucosum* and *P. nordicum* [2–8]. OTA has been shown to be nephrotoxic, carcinogenic, teratogenic, and immunosuppressive [3, 9–11]. In addition, OTA has been shown to induce oxidative DNA damage in mammalian cells and in rat liver and kidney in vivo, indicating its role in cytotoxicity and tumorigenicity [12, 13]. Food products associated with OTA contamination include cereal grains, beer, wine, grapes, coffee, and dried fruit and nuts [2, 3, 14–20]. As a result of the wide variety of sources of this toxin, regulatory limits for OTA content in commodities have been set in the European Union [21].

Fungal production of OTA has been shown to be affected by environmental and nutritional factors, such as pH, temperature, water activity, and carbon and nitrogen sources [22–25]. Food additives including sodium propionate, methyl paraben, sodium

bisulfite, and potassium sorbate were shown to inhibit OTA production in *A. sulphureus* [26], but there have been no other studies involving the effects of chemical natural products on OTA biosynthesis. In addition, because the biochemical and genetic components of the OTA biosynthetic pathway is not fully understood, and because of the species diversity of OTA producers, molecular studies regarding the regulation of OTA production have been limited [24, 25].

Recently, several antioxidant compounds have been shown to inhibit aflatoxin biosynthesis in *A. flavus* and *A. parasiticus* [27–30], and several genetic mechanisms involving oxidative stress-responsive genes have been identified [31, 32]. We hypothesized that OTA production in ochratoxigenic *Aspergillus* species would be affected similarly by phenolic antioxidants. Using a defined medium, the effects of these compounds on OTA production and fungal growth were investigated, as a first step in determining whether ochratoxigenesis is dependent on similar biochemical and genetic mechanisms as aflatoxigenesis.

Materials and methods

Ochratoxigenic fungal strains

A. alliaceus NRRL315 (isolated from blister beetle), *A. albertensis* NRRL20602 (isolated from human ear), *A. melleus* NRRL3520 (of unknown origin), *A. sulphureus* NRRL4077 (isolated from soil), and *A. carbonarius* NRRL369 (isolated from paper) were obtained from the USDA Agricultural Research Service (NRRL) Culture Collection (Peoria, IL). *A. ochraceus* ATCC22947 (isolated from sorghum) was obtained from the American Type Culture Collection (Manassas, VA). Other fungal strains used in this study were isolated previously from different sources (P. Bayman and J. Baker, unpublished). *A. alliaceus* O-106 was isolated from a Joshua tree (*Yucca brevifolia*) flower. *A. lanosus* strains 114 and 171 and *A. sclerotiorum* O-196 were isolated from pistachio orchard soil. *A. ochraceus* R46 was isolated from coffee borer beetle. *A. elegans* J-93 was isolated from green coffee. Strains were grown on potato dextrose agar (PDA) (Difco, Becton Dickinson, Franklin Lakes, NJ) at 28°C until conidia developed, and conidial stock suspensions were made in 30% glycerol, 0.05% Tween 80 for storage at –70°C.

Media and growth conditions

Experiments were performed using *Aspergillus* minimal medium (AMM) [33] containing 100 mM glucose and 70 mM NH_4Cl as carbon and nitrogen sources, respectively. For *A. ochraceus* strains, AMM was supplemented with 1% (wt vol^{-1}) yeast extract. Gallic acid (3,4,5-trihydroxybenzoic acid), vanillic acid (4-hydroxy-3-methoxybenzoic acid), protocatechuic acid (3,4-dihydroxybenzoic acid), 4-hydroxybenzoic acid, (+) catechin, caffeic acid (3,4-dihydroxycinnamic acid), and chlorogenic acid (all from Sigma-Aldrich, St. Louis, MO) were added individually to AMM and the media were sterilized by filtration using 0.2 µm cellulose nitrate filters (Nalge Nunc International, Rochester, NY). Fungal cultures were incubated at 28°C without shaking.

Effect of antioxidant concentration on OTA production by *A. alliaceus*

AMM was prepared with 0, 1.25, 2.5, 5, 10, or 20 mM of each antioxidant compound, and 1-ml aliquots were dispensed in triplicate into 24-well culture plates. Wells were inoculated with 10 µl of *A. alliaceus* strain O-106 conidial suspension ($\sim 10^5$ conidia ml^{-1}) and incubated at 28°C for 5 days. OTA in 100 µl of each culture was partitioned into 100 µl of chloroform, which was dried under a stream of nitrogen gas and redissolved in 10 µl of methanol. Samples (1 µl each) were spotted onto 0.5-mm-thick silica gel thin-layer chromatography (TLC) plates and separated using a mobile phase of toluene:ethyl acetate:formic acid (5:4:1). OTA spots were visualized under ultraviolet light in comparison to authentic OTA (Sigma-Aldrich), and spot intensities were determined using ImageJ image analysis software [34]. Mean OTA content in triplicate cultures of each treatment was calculated relative to untreated triplicate cultures.

Quantification of OTA production and growth of *Aspergillus* species

Each antioxidant compound was added to AMM at a concentration of 10 mM. Ten ml of each medium were added to 60 mm diameter Petri dishes and inoculated with 10 µl of conidial suspensions ($\sim 10^5$–10^6 conidia ml^{-1}) of each *Aspergillus* strain

described above. Plates were sealed with parafilm and incubated at 28°C for 6 days. Following incubation, 3 ml of culture liquid from each plate were filtered using 0.22 μm Millex-GP polyethersulfone syringe filters (Millipore, Bedford, MA). OTA in the culture filtrates was partitioned into 3 ml of chloroform. The chloroform phase was collected, evaporated to dryness under a stream of nitrogen gas and redissolved in 1 ml of HPLC-grade methanol. Samples were analyzed by injection of 20 μl into an Agilent model 1100 high-performance liquid chromatograph with model 1321A fluorescence detector (Agilent Technologies, Inc., Santa Clara, CA). Separations were run on a Vydac 218TP54, 4.6 × 250 mm C_{18} column (Grace Vydac, Hesperia, CA) with methanol:water:phosphoric acid (70:30:0.1) as the mobile phase at a flow rate of 1 ml min^{-1}. OTA was detected using excitation and emission wavelengths of 333 nm and 418 nm, respectively. OTA amounts per sample (3 ml culture filtrate) were calculated from peak areas in comparison to a standard curve constructed using 5–300 ng of authentic OTA (Sigma-Aldrich) per injection. Total OTA per plate was calculated as OTA per sample × 3.33. From the same cultures, fungal mycelium was recovered by filtration onto Whatman no. 4 paper filters and air dried overnight for dry weight determination. Specific OTA production was calculated as ng OTA/mg fungal dry weight.

Statistical analyses

All treatments for quantification were performed in triplicate. Data were analyzed using GraphPad InStat version 3.06 (GraphPad Software, San Diego, CA). One-way ANOVA with Dunnett's posttest was performed to compare specific OTA production and fungal dry weights in antioxidant-treated samples to untreated samples.

Results and discussion

Previous efforts in our laboratory to understand OTA production focused on California isolates of *A. alliaceus.* Initial experiments showed that OTA production by *A. alliaceus* strain O-106 was inhibited in response to increasing concentrations of phenolic antioxidants. As shown in Table 1, most of the compounds inhibited the total OTA content relative to control cultures at concentrations of 10 mM or higher, with the exception of catechin. We therefore tested these compounds for OTA inhibition in other *Aspergillus* species at 10 mM.

Table 2 shows the effects of the antioxidant compounds we tested on OTA production by nine OTA-producing *Aspergillus* species. In the 12 strains tested, OTA content of untreated cultures was highly variable among triplicate samples, leading to large standard deviations, which in some cases precluded meaningful statistical analyses of antioxidant treatments relative to controls. This variability has been attributed by other researchers investigating OTA production to medium effects [23], and our own observations with OTA suggest that minimizing variability requires more thorough investigation of potential causes. These challenges are also inherent in

Table 1 Antioxidant effects on OTA production in *A. alliaceus* O-106[a]

Treatment	Antioxidant concentration (mM)					
	0	1.25	2.5	5	10	20
Gallic acid	100 (26)	150 (11)	134 (4)	128 (2)	68 (26)	74 (18)
Vanillic acid	100 (16)	154 (2)	134 (5)	59 (6)	n.d.[b]	n.d.
Protocatechuic acid	100 (14)	117 (4)	128 (5)	115 (2)	65 (5)	24 (10)
4-Hydroxybenzoic acid	100 (6)	100 (6)	92 (7)	71 (30)	30 (5)	16 (2)
Catechin	100 (12)	130 (25)	134 (7)	124 (46)	143 (4)	97 (26)
Caffeic acid	100 (15)	133 (3)	118 (8)	101 (18)	52 (17)	28 (5)
Chlorogenic acid	100 (3)	83 (4)	79 (3)	70 (10)	25 (6)	16 (6)

[a] Mean OTA production per fungal culture, expressed as percent relative to untreated cultures. Numbers in parentheses represent standard deviation of three replicate treatments

[b] n.d.—not detected

Table 2 Effect of phenolic antioxidants on OTA production in *Aspergillus* spp[a]

Treatment	*A. alliaceus* NRRL315	*A. alliaceus* O-106	*A. lanosus* 114	*A. lanosus* 171	*A. ochraceus* ATCC22947	*A. ochraceus* R46
Control	118 (73.3)	67.3 (35.0)	64.7 (40.4)	78.8 (38.0)	107 (35.1)	642 (143)
Gallic acid	44.7 (27.1)	14.3 (4.7)[b]	n.d.[b,c]	0.7 (0.5)[b]	199 (222)	378 (26.1)
Vanillic acid	22.9 (39.6)[b]	1.6 (0.6)[b]	n.d.[b]	n.d.[b]	143 (204)	732 (978)
Protocatechuic acid	79.1 (51.2)	25.8 (10.5)[b]	0.3 (0.2)[b]	1.4 (0.8)[b]	418 (363)	125 (130)
4-Hydroxybenzoic acid	n.d.[b]	5.1 (2.7)[b]	n.d.[b]	n.d.[b]	345 (397)	798 (556)
Catechin	71.0 (16.3)	53.4 (2.6)	24.6 (7.3)[b]	15.8 (5.2)[b]	19.7 (16.7)	14.5 (8.1)
Caffeic acid	10.0 (4.8)[b]	13.1 (10.4)[b]	n.d.[b]	1.6 (0.2)[b]	344 (292)	293 (54.2)
Chlorogenic acid	29.5 (5.7)[b]	6.5 (1.3)[b]	0.1 (0.2)[b]	0.6 (0.3)[b]	95.7 (113)	110 (98.2)
Treatment	*A. albertensis* NRRL20602	*A. melleus* NRRL3520	*A. sulphureus* NRRL4077	*A. carbonarius* NRRL369	*A. elegans* J-93	*A. sclerotiorum* O-196
Control	184 (91.2)	239 (84.4)	150 (36.6)	19.3 (16.5)	151 (43.7)	641 (313)
Gallic acid	60.5 (32.1)[b]	46.1 (22.7)	n.d.[b]	3.5 (4.2)	n.d.[b]	1.8 (0.7)[b]
Vanillic acid	n.d.[b]	13.5 (7.6)	n.d.[b]	25.3 (30.5)	n.d.[b]	n.d.[b]
Protocatechuic acid	33.1 (19.7)[b]	35.8 (0.7)	2.4 (2.6)[b]	16.1 (13.8)	0.2 (0.0)[b]	26.7 (9.6)[b]
4-Hydroxybenzoic acid	6.2 (2.3)[b]	7.5 (5.8)	n.d.[b]	1.5 (1.1)	1.7 (0.4)[b]	17.2 (29.8)[b]
Catechin	18.6 (6.6)[b]	1580 (296)[d]	0.4 (0.2)[b]	29.6 (12.9)	62.0 (105)	5.5 (3.3)[b]
Caffeic acid	31.8 (10.6)[b]	18.2 (6.3)	n.d.[b]	9.2 (14.3)	n.d.[b]	6.4 (1.5)[b]
Chlorogenic acid	26.4 (7.0)[b]	563 (186)[d]	6.3 (9.6)[b]	6.3 (7.4)	n.d.[b]	136 (50.6)[b]

[a] Mean OTA content, expressed as ng OTA/mg fungal dry weight. Numbers in parentheses represent standard deviation of three replicate samples per treatment

[b] Significant reduction of OTA content relative to control, $P < 0.05$

[c] n.d.—not detected

[d] Significant increase in OTA content relative to control, $P < 0.05$

analyses of aflatoxin production by different *A. flavus* strains (R. Molyneux and N. Mahoney, personal communication). Nevertheless, the data indicate consistent trends of OTA inhibition among treatments, which are illustrated in the ranges of inhibition among triplicate samples.

OTA production by *A. alliaceus* strains NRRL315 and O-106 was inhibited strongly by 4-hydroxybenzoic acid (94–100%), caffeic acid (63–96%), and chlorogenic acid (70–90%). Vanillic acid (42–100%), protocatechuic acid (40–74%), and gallic acid (36–86%), also showed OTA inhibition in both *A. alliaceus* strains. Catechin (17–56%) was generally less effective at inhibiting OTA production in these strains. Similarly, *A. albertensis* exhibited strong OTA inhibition, most effectively by vanillic acid (100%) and 4-hydroxybenzoic acid (96–98%), followed by catechin (87–94%), chlorogenic acid (83–90%), caffeic acid (79–89%), protocatechuic acid (72–93%), and gallic acid (57–87%).

OTA production by *A. lanosus* strains 114 and 171 was inhibited strongly by all the compounds tested. Other than catechin, which inhibited toxin production by 52–87%, all the other compounds inhibited 97–100% of the toxin production by these strains. These effects were also shown in the *A. sulphureus* and *A. sclerotiorum* strains tested, showing 88–100% and 74–100% OTA inhibition, respectively. In *A. elegans*, OTA production was inhibited by all the compounds tested by 99–100%, with the exception of a single catechin treatment that showed OTA production at the level of the control treatments.

A. melleus showed more variable inhibition by antioxidant compounds. Vanillic acid (91–97%), 4-hydroxybenzoic acid (94–99%), and caffeic acid (90–95%) tended to inhibit OTA production most strongly. Intermediate levels of inhibition were seen for protocatechuic acid (85%) and gallic acid (70–88%). In contrast, *A. melleus* cultures grown on AMM containing catechin produced 5- to 8-fold more

OTA than control cultures, and on AMM containing chlorogenic acid produced 1.6- to 3-fold more OTA than control cultures.

The effects of the antioxidant compounds on *A. carbonarius* were also highly variable. Although the results were not statistically significant, gallic acid, 4-hydroxybenzoic acid, and chlorogenic acid tended to inhibit OTA production. In contrast, vanillic acid, caffeic acid, protocatechuic acid, and catechin produced widely divergent effects among replicate samples, ranging from 97% OTA inhibition to 3-fold higher OTA levels relative to control treatment in individual samples. It should be noted that in AMM containing glucose and ammonium, control cultures of *A. carbonarius* produced very low levels of OTA relative to the other strains tested in this study, which may contribute to the high variability in its response to antioxidant treatments.

In contrast to the other strains tested in this study, *A. ochraceus* strains ATCC22947 and R46 grew slowly and did not produce OTA on AMM containing glucose and ammonium (data not shown). Therefore, yeast extract was added to the media for growth of these strains, to promote OTA production and more vigorous growth. However, this tended to increase the variability in OTA production among replicate samples. Although treatments were not significantly different, trends among treatments were apparent, as shown in Table 2. *A. ochraceus* strain R46 produced higher levels of OTA than *A. ochraceus* strain ATCC22947. Also, antioxidant compounds generally were more inhibitory to OTA production in *A. ochraceus* strain R46 than in *A. ochraceus* strain ATCC22947. Catechin, chlorogenic acid, and protocatechuic acid tended to strongly inhibit OTA production in strain R46. Lower amounts of OTA inhibition were seen with caffeic acid and gallic acid. In contrast, the response of strain R46 to vanillic acid and 4-hydroxybenzoic acid was inconsistent among replicates, with a range of 82% inhibition to 3-fold increase and 53% inhibition to 2-fold increase, respectively, in OTA production. *A. ochraceus* strain ATCC22947 generally showed no consistent OTA inhibition in response to any of the antioxidants tested, with the exception of catechin, which inhibited OTA production by 70–99%. The differences in OTA levels produced by control cultures and response to antioxidants between *A. ochraceus* strains may be due in part to laboratory domestication [7], i.e., strain ATCC22947 was isolated in 1972 [35] and has been subcultured many times, while strain R46 was isolated in 2002 and subcultured only a few times. Also, since OTA production generally is higher on complex media, growth of *A. ochraceus* on AMM supplemented with yeast extract may have made the inhibitory effects of the compounds difficult to discern.

In addition to the effects on OTA production, antioxidant compounds also affected fungal growth (Table 3). *A. alliaceus* strain NRRL315 was inhibited most strongly by vanillic acid and 4-hydroxybenzoic acid, and significant effects on growth were seen for all other compounds except catechin. In contrast, growth of *A. alliaceus* strain O-106 was inhibited only by vanillic acid. Interestingly, in strain O-106, 4-hydroxybenzoic acid, chlorogenic acid, caffeic acid, and gallic acid inhibited OTA production (Table 2) with no inhibition of fungal growth (Table 3). As with *A. ochraceus* strains, strain differences in this case may be due to the laboratory domestication of *A. alliaceus* strain NRRL315 and the relative wildness of strain O-106.

Growth was inhibited in both *A. lanosus* strains most strongly by vanillic acid and 4-hydroxybenzoic acid. Like *A. alliaceus* strain O-106, *A. lanosus* growth was less affected by the other antioxidants tested (although the reduction was statistically significant), at the same time OTA production was strongly inhibited. Similarly, gallic acid and chlorogenic acid did not affect growth of *A. carbonarius* but tended to inhibit OTA production. In *A. elegans*, treatment with gallic acid, 4-hydroxybenzoic acid, and caffeic acid inhibited OTA production without affecting growth, and treatment with protocatechuic acid and chlorogenic acid resulted in significantly higher biomass than controls and strong inhibition of OTA production.

Growth of *A. sulphureus* was significantly reduced by all compounds tested except catechin, which increased fungal biomass significantly while inhibiting OTA production. Similarly, *A. sclerotiorum* growth was significantly inhibited by gallic acid, vanillic acid, 4-hydroxybenzoic acid, and chlorogenic acid, which also inhibited OTA production. OTA production, but not growth, was inhibited by catechin, while caffeic acid affected OTA production more strongly than growth, although growth inhibition was significant. *A. albertensis* growth was reduced only by vanillic acid and 4-hydroxybenzoic acid, which were also the strongest inhibitors of OTA production. *A. melleus* growth was affected by gallic

Table 3 Effect of phenolic antioxidants on growth of *Aspergillus* spp[a]

Treatment	*A. alliaceus* NRRL315	*A. alliaceus* O-106	*A. lanosus* 114	*A. lanosus* 171	*A. ochraceus* ATCC22947	*A. ochraceus* R46
Control	60.5 (6.9)	60.3 (4.4)	61.4 (5.4)	62.0 (4.7)	195.2 (6.3)	199.5 (2.1)
Gallic acid	32.7 (8.9)[b]	63.6 (1.3)	57.7 (6.6)	49.1 (5.6)[b]	202.7 (8.7)	210.6 (5.4)
Vanillic acid	1.6 (0.2)[b]	17.8 (2.7)[b]	7.8 (2.5)[b]	6.4 (0.2)[b]	134.4 (41.1)[b]	139.4 (39.2)[b]
Protocatechuic acid	45.6 (4.8)[b]	61.5 (3.3)	47.5 (1.8)[b]	49.8 (8.0)[b]	191.1 (20.6)	189.7 (24.1)
4-Hydroxybenzoic acid	2.6 (1.1)[b]	54.5 (2.1)	8.0 (0.6)[b]	7.9 (0.4)[b]	184.0 (15.8)	206.3 (16.0)
Catechin	63.5 (1.7)	73.6 (5.2)[c]	67.0 (3.4)	66.9 (3.4)	220.4 (2.6)	197.0 (35.3)
Caffeic acid	29.1 (9.5)[b]	59.8 (1.7)	56.9 (1.2)	49.1 (3.3)[b]	195.7 (14.5)	221.2 (1.7)
Chlorogenic acid	21.6 (5.9)[b]	68.2 (3.0)[c]	47.7 (4.4)[b]	43.1 (7.4)[b]	218.3 (11.9)	240.6 (11.4)
Treatment	*A. albertensis* NRRL20602	*A. melleus* NRRL3520	*A. sulphureus* NRRL4077	*A. carbonarius* NRRL369	*A. elegans* J-93	*A. sclerotiorum* O-196
Control	65.4 (4.1)	35.3 (2.6)	25.2 (3.3)	109 (1.3)	23.3 (0.4)	40.0 (3.3)
Gallic acid	72.0 (9.7)	26.2 (7.1)[b]	1.1 (0.5)[b]	109 (10.6)	28.8 (3.4)	6.2 (0.6)[b]
Vanillic acid	4.5 (1.2)[b]	20.4 (2.0)[b]	1.6 (0.5)[b]	90.2 (28.4)	12.8 (0.5)[b]	4.9 (0.4)[b]
Protocatechuic acid	70.7 (7.1)	30.1 (1.7)	2.1 (0.9)[b]	112 (4.8)	40.4 (3.0)[c]	36.0 (3.1)
4-Hydroxybenzoic acid	25.5 (5.5)[b]	30.7 (2.1)	3.1 (0.8)[b]	67.8 (9.3)[b]	31.3 (2.6)	5.5 (0.8)[b]
Catechin	74.9 (5.3)	30.9 (3.3)	34.3 (1.3)[c]	112 (13.4)	8.6 (5.3)[b]	37.7 (5.0)
Caffeic acid	65.1 (2.9)	35.9 (1.0)	11.6 (3.2)[b]	100 (11.7)	30.6 (4.0)	31.1 (2.4)[b]
Chlorogenic acid	64.0 (6.9)	26.7 (2.1)[b]	5.1 (0.8)[b]	120 (15.0)	41.6 (7.1)[c]	9.0 (2.2)[b]

[a] Mean fungal growth, expressed as mg dry weight. Numbers in parentheses represent standard deviation of three replicate samples per treatment

[b] Significant reduction of fungal dry weight relative to control, $P < 0.05$

[c] Significant increase in fungal dry weight relative to control, $P < 0.05$

acid, vanillic acid, and chlorogenic acid, but not by OTA-inhibiting 4-hydroxybenzoic acid.

Growth of *A. ochraceus* strains in AMM supplemented with yeast extract was significantly inhibited by vanillic acid, but not by the other compounds tested. *A. ochraceus* strain ATCC22947 showed similar results in AMM without yeast extract, while growth of strain R46 was reduced by vanillic acid, protocatechuic acid, 4-hydroxybenzoic acid, and catechin in AMM without yeast extract (data not shown). Differences in growth response to antioxidant compounds between *A. ochraceus* strains, like differences in OTA production, may be in part due to differences in laboratory domestication, as discussed above.

Conclusions

Data presented in this study indicate that antioxidants generally suppress OTA production in several important ochratoxigenic *Aspergillus* species. All of the antioxidant compounds tested in this study were inhibitory to one or more *Aspergillus* strain tested, and vanillic acid, and 4-hydroxybenzoic acid were the most effective inhibitors of OTA production and growth of many of these strains. These data suggest that the mechanisms of phenolic antioxidant activity may be directly or indirectly related to primary metabolism, as evidenced by effects on fungal growth, or involved in secondary metabolism, or a combination of the two. Further experimentation will be necessary to determine whether the effects on production of secondary metabolites, such as OTA are due to effects on the oxidative stress response of the fungus. Also, OTA inhibition by these compounds may be useful in determining differences in gene expression in response to the compounds, and to identify biosynthetic and regulatory genes necessary for OTA production. Additional studies to determine dose-response effects of inhibitory compounds on OTA production in a broad range of ochratoxigenic *Aspergillus* species will be useful in indicating

effective inhibitory concentrations for potential use in preventative intervention strategies.

Phenolic antioxidant compounds have been studied in coffee [36], grapes and wine [37], barley [38, 39], and tree nuts including walnut [28, 40], almond [41, 42], and pistachio [43, 44]. These antioxidant sources are also common habitats of ochratoxigenic *Aspergillus* and *Penicillium* species, leading to potential OTA contamination of these commodities. This apparent contradiction indicates that the role of antioxidants and OTA production in the ecology of ochratoxigenic fungi is complex. Our results show that individual antioxidants have variable effects on both OTA production and growth of individual ochratoxigenic *Aspergillus* strains and that these effects may or may not be independent of each other. As a result, further investigations regarding bioavailability and fungal response to phenolic and polyphenolic antioxidants under ecologically relevant conditions is warranted. Information regarding genetic and physiological responses to antioxidant compounds could lead to targeted intervention strategies for the reduction of economic losses by OTA contamination.

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